Persistent Organic Pollutants and Endothelial Cell Dysfunction: Implications in Vascular Diseases

Elizabeth Oesterling[1], X. Arzuaga[2], Z. Majkova[1], G. Reiterer[3], M. Toborek[3,4], B. Hennig[1,2,3]

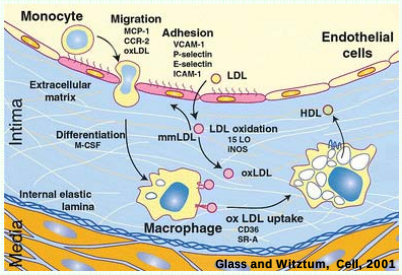
[1]Graduate Center for Toxicology, University of Kentucky, Lexington KY; [2]Molecular and Cell Nutrition Laboratory, College of Agriculture, UK; [3]Graduate Center for Nutritional Sciences, UK; [4]Department of Surgery, UK

Background

Cardiovascular disease is the number one killer in the United States

Three-fourths of cardiovascular deaths can be attributed to the inflammatory disease atherosclerosis, in which endothelial activation is an initiating event. The vascular endothelium is uniquely susceptible to physiological insult due to its constant contact with circulating xenobiotics and inflammatory mediators. Persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) like benzo[a]pyrene (BP) and fluoranthene, are xenobiotics that can lead to endothelial cell dysfunction and pro-inflammatory responses.

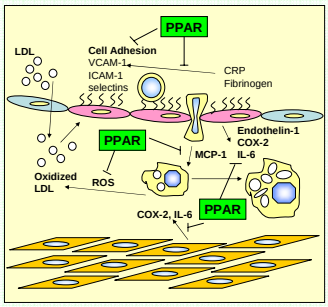
Early Events in Atherosclerosis



Endothelial cell dysfunction is considered an inaugural event in the formation of an atherosclerotic plaque. Up-regulation of adhesion molecules such as intracellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) will lead to the attachment and buildup of immune cells on the endothelial wall. This endothelial activation leads to the release of pro-inflammatory cytokines such as cyclooxygenase-2 (COX-2) and interleukin-6 (IL-6). The endothelium becomes more permeable to lipid particles and immune cells, leading to the production of foam cells.

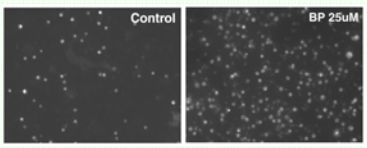
PPAR Signaling

Peroxisome proliferator-activated receptor is a nuclear receptor that is highly expressed in the heart. It may exert direct antiatherogenic actions in the vascular wall by regulating expression of a number of key proteins involved in atherogenesis, inflammation, plaque instability, and thrombosis.



Results

Benzo[a]pyrene leads to increased monocyte adhesion to the endothelium



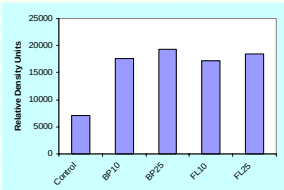
Human endothelial cells were treated with benzo[a]pyrene (25 μM) for 6 hours. Monocytes were activated with benzo[a]pyrene (25 μM) for 10 minutes and then loaded with the fluorescent probe, calcein. Fluorescently labeled macrophages were added to the endothelial layer for 30 min, fixed with glutaraldehyde, and visualized by fluorescent microscope.

Results

Benzo[a]pyrene and Fluoranthene increase ICAM-1 protein production

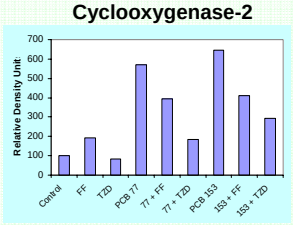
Human endothelial cells were pre-treated with aryl hydrocarbon receptor (AhR) agonist beta-naphthoflavone for 16 hours, then treated with either DMSO, benzo[a]pyrene (10 or 25 μM), or fluoranthene (10 or 25 μM). Total cellular protein was extracted and analyzed for ICAM expression by immunoblot.

Intracellular Adhesion Molecule-1

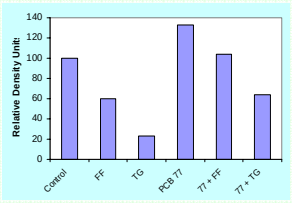


PCB 77 and 153 induction of COX-2, IL-6, VCAM-1, and CYP1A1 can be rescued by PPAR agonists

Cyclooxygenase-2

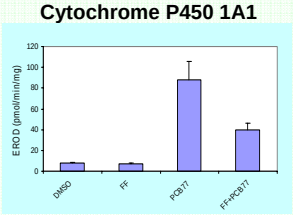


Interleukin-6

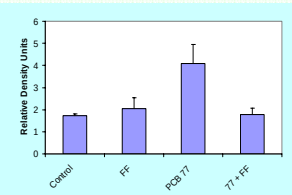


Porcine primary endothelial cells were pretreated with PPAR-alpha agonists, fenofibrate (FF, 10 μM), or PPAR-gamma agonists, thiazolidinedeione (TZD, 25 μM) or troglitazone (TG, 25 μM) and exposed to PCB 153 and 77 (3.4 μM) for 4 h to measure COX-2 expression or 6 hours for IL-6 expression. Total cellular RNA was extracted followed by RT-PCR. PCR products were separated on 2% agarose gel and stained with SYBR gold. β-actin was used as a housekeeping gene.

Cytochrome P450 1A1



Vascular Adhesion Molecule-1



Porcine primary endothelial cells were co-treated with the PPAR-alpha ligand fenofibrate (FF, 100 μM) and PCB77 (3.4 μM) for 18 hrs before ethoxyresorufin –O-deethylase (EROD) measurement. EROD activity was measured using the CYP1A substrate 7-ethoxyresorufin.

Porcine primary endothelial cells were co-treated with the PPAR-alpha ligand fenofibrate (FF, 10 μM) and PCB77 (3.4 μM) for 24 hrs before protein extraction and VCAM1 measurement by immuno blot. β-actin was used as a housekeeping gene.

Conclusions

Results suggest that PCBs and PAHs can lead to endothelial cell activation and dysfunction, and that PPAR agonists may provide protection by down-regulating POP-induced pro-atherogenic inflammatory events.

This research is supported by the Superfund Basic Research Program at the University of Kentucky , NIEHS/NIH 5 P42 ES 007380, the American Heart Association (0215075B), and an NIEHS Training Grant T32 ES 07266

NIEHS National Institute of Environmental Health Sciences

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

epascienceforum 2006 Your Health • Your Environment • Your Future